AGGREGATION OF BASIC REGULAR BLOOD ELEMENTS IN CALVES DURING THE PHASE OF MILK FEEDING

ABSTRACT

The aim was to find out aggregation activity of regular blood elements of calves during the phase of milk feeding. The study was conducted in Kolos farm of Fatezh district in Kursk region, Russia, spring of 2014. The study used 39 calves of black and white breed, taken into investigation on the 11th day of life. Examination was made on the 11th, 15th, 20th, 25th and 30th days of calves' life with the usage of biochemical, hematological and statistical methods of investigation. We estimated the intensity of lipids' peroxidation in plasma, aggregation of erythrocytes, platelets and neutrophils. During the phase of milk feeding the calves were noted to have a tendency to the increase of spontaneous aggregation of erythrocytes. It could be judged by a light tendency for the increase of summary quantity of erythrocytes in an aggregate, quantity rise of aggregates themselves and number lowering of disaggregated erythrocytes. All the calves during milk feeding were noted to have a tendency to strengthening of platelets' aggregation. So, on the 11th day of life their period of platelets' aggregation development under collagen impact was equal to 30.7±0.12s, decreasing to some extent during investigation. Analogical platelets' aggregation state of healthy animals was noted for adenosine diphosphate (to the end of the phase 38.1±0.15s) and ristomicin (to the end of the phase 46.2±0.17s). In later period there developed thrombin and adrenaline platelets' aggregation, also having a tendency to light acceleration during investigation and being equal to its end to 51.3±0.18s and 98.0±0.34s, respectively. During the phase of milk feeding the calves were also noted to have a little tendency to strengthening of neutrophils' aggregation. So, their neutrophils' aggregation during investigation rose with lectin on 4.6%, with concanavalin A - on 6.4%, with phytogemagglutinin - on 3.2%. During the phase of milk feeding the calves were noted to have a little tendency to strengthening of lipids' peroxidation in plasma. The calves of the age between 11 to 30 days of life were found to have little strengthening of regular blood elements' aggregation.

Key words: phase of milk feeding, calves, aggregation, erythrocytes, platelets, white blood cells.

1. INTRODUCTION

Blood, consisting of regular elements and plasma, continuously circulates along vessels in a living body [1]. It provides gas exchange and delivery of nutrients and biologically active substances to tissues [2,3], and also removal of metabolic waste products out of them [4,5]. The efficiency of hemocirculation, especially in microcirculation system, mostly depends on regular blood elements' aggregation [6,7]. Its evidence is under constant control from the side of a vascular wall [8,9]. It was noted that surplus aggregation of erythrocytes, platelets and leucocytes can inhibit metabolic processes in a body [10,11]. In this connection we find to be very urgent the estimation of the degree of regular blood elements' aggregation in calves at the beginning of their ontogenesis - in the phase of milk feeding [12]. Given investigations are important for both fundamental science and practice as abnormalities in the processes of aggregation and disaggregation in blood play essential role in pathogenesis of many diseases [13,14]. Both physiology of animals and veterinary science need precisely adjusted normative indices of basic regular blood elements' aggregation [15]. These norms are necessary for the estimation of dynamics of cattle state, including calves of milk feeding in case of application of various impacts on their bodies [16].

The following aim was put in our investigation - to find out aggregation activity of regular blood elements in calves during the phase of milk feeding.

2. MATERIALS AND METHODS

The research was conducted in strict accordance with ethical principles established by the European Convent on protection of the vertebrata used for experimental and other scientific purposes (adopted in Strasbourg in March 18, 1986, and confirmed in Strasbourg in June 15, 2006) and approved by the local ethic committee of Kursk Institute of Social Education, branch of Russian State Social University (Record №12 dated December 3, 2015) and the local ethic committee of All-Russian SII of Physiology, Biochemistry and Animals' feeding (Record №11, dated December 4, 2015).

The study used 39 calves of black-many coloured breed, taken into investigation on the 11th day of life. All the calves were got in autumn. The animals were kept in Kursk region (Central Russia) in calf-sheds of the farm “Kolos” without special heating. They received whole milk for feeding in quantity which was necessary for them. Examination was made five times during the phase of milk feeding - on the 11th, 15th, 20th, 25th and 30th days of life.

Comment [AL1]: If the animals were fed from the bucket, please highlite the exact amount of milk as percent of body weight? If the animals were kept with their dams give that information in the manuscript.

The activity of the processes of lipids' peroxidation (LPO) in plasma was estimated by the content of thiobarbituric acid (TBA)-active products with the help of a set "Agat-Med" and acyl hydroperoxides (AHP). Antioxidant potential of liquid part of blood was defined by its antioxidant activity (AOA) [17].

The evidence of erythrocytes' aggregation was defined with the help of a light microscope in Gorjaev's box. We registered the quantity of erythrocytes' aggregates, the number of aggregated and disaggregated erythrocytes [18].

Platelets' aggregation (AP) was estimated with the help of visual micromethod of AP estimation [19] with the usage of adenosine diphosphate (ADP) (0.5×10^{-4} M), collagen (dilution 1:2 of basic suspension), thrombin (0.125 un/ml), ristomicin (0.8 mg/ml) and adrenaline (5.0×10^{-6} M) in rich in platelets plasma with standardized platelets' quantity 200×10^{9} tr. Activity of neutrophils' aggregation was estimated with the help of a photoelectrocolorimeter. As inductors we used lectin of wheat foetus in a dose of 32 mkg/ml, concanavalinA - 32 mkg/ml and phytogemagglutinin - 32 mkg/ml.

Statistical processing of received data was made with the help of a programme package “Statistics for Windows v. 6.0”, “Microsoft Excel”. The results were processed by Student's criterion (t). Differences in data were considered reliable in case of $p<0.05$.

Comment [AL2]: Please use the different statistical method and provide the details about the statistical model

3. RESULTS AND DISCUSSION

Examined calves were noted to have small LPO activity of plasma with a slight tendency to strengthening during the period of investigation - the content of AHP in it rose from 1.44±0.17 D_{233}/1ml to 1.47±0.25 D_{233}/1ml, TBA-active products - from 3.59±0.15 umol/lto 3.64±0.28 umol/l. It was accompanied by a tendency to some weakening of plasma AOA from 33.5±0.38% on the 11th day of life to 33.0±0.34% on the 30th day of calves' life.

During the phase of milk feeding the calves were noted to have unexpressed tendency in the rise of spontaneous erythrocytes' aggregation. It could be judged by a slight tendency to the increase of summary erythrocytes' quantity in an aggregate (on 1.9%), quantity rise of aggregates themselves (on 2.4%) and number lowering of disaggregated erythrocytes (on 2.2%) (table).

All the calves during milk feeding were noted to have a tendency to strengthening of platelets' aggregation. So, on the 11th day of life their period of AP development under the impact of collagen was equal to 30.7±0.12s, decreasing to some extent during investigation. Analogical AP state of healthy animals was noted for ADP (to the end of the phase - 38.1±0.15s) and ristomicin (to the end of the phase - 46.2±0.17s). In later period there developed thrombin and adrenaline AP, having also a tendency to light acceleration during investigation and being equal to its end to 51.3±0.18s and 98.0±0.34s, respectively (table).

During the phase of milk feeding the calves were also noted to have a small tendency to strengthening of neutrophils' aggregation. So, during investigation their neutrophils' aggregation rose with lectin on 4.6%, with concanavalinA - on 6.4%, with phytogemagglutinin - on 3.2% (table).

The consumption of milk and beef by the population of the planet increases, and it dictates the necessity of constant development of this agricultural branch. It can be achieved in the result of continuation of active scientific investigations of cattle physiology [15]. In this connection special significance is given to investigations of calves' blood physiology at the beginning of ontogenesis [20]. So, great attention is devoted to investigations of calves preparing for the beginning of vegetable feeds' consumption. In our work the calves were noted to have gradual strengthening of plasma AOA at the age between the 11th and 30th days of life which was accompanied by gradual weakening of LPO activity. Found facts were supported by the results of earlier investigations [21]. It is known that intensity of freely-radical processes in plasma influences significantly the morphofunctional state of erythrocytes, platelets and leucocytes [22,23]. It can explain the slight ability in aggregation of basic regular blood elements in calves during the phase of milk feeding.

In the carried-out work special attention is paid to aggregation of uniform elements of blood. Intra vascular formation of units and success of microcirculation in many respects depends on her level. In this regard processes of a metabolism and intensity of growth at animals depend on activity of aggregation of uniform elements of blood.

It is evident that the great quantity of electronegative proteins on the surface of erythrocytes [24] mostly lies in the basis of small activity of erythrocytes' aggregation in calves at milk feeding. High control over generation of oxygen active forms in calves provides minimization of oxidative damages of membrane erythrocyte proteins and globular plasma proteins, participating in aggregation [25]. In this connection we can consider that the phase of milk feeding of calves is characterized by optimum of metabolic and receptor processes in erythrocytes. Received estimation results of erythrocytes' aggregation are confirmed by the single work containing information about the tendency to its strengthening in calves of the given age [26]. We should compare received results with literature data with great caution as in the previous work the groups were mixed, as far as breed is concerned, but calves of Simmental breed prevailed. Besides, they were got in autumn, and it also makes comparison of results difficult.

Noted in calves during the phase of milk feeding tendency to strengthening of platelets' aggregative activity is connected with activity increase of their receptors and postreceptor mechanisms of aggregation. Evidently, in calves' blood at the age of 11-30 days gradually rises concentration of von Willebrand Factor - cofactor of platelets' adhesion. It is accompanied by weak number increase of receptors to it - (GPIb) on platelets' surface. It was pointed in calves by a tendency to decrease of AP period in response to ristomicin. Found AP dynamics in response to strong and weak agonists of aggregation can be explained by physiologically approved activity changes of platelet phospholipase A_2 and C. They provide functioning of thromboxan and phosphoinositol ways of platelets' activation [27,28]. In literature there is rather poor information about platelets' activity in calves of milk feeding [29]. In spite of the fact that famous sources confirm that calves of milk feeding have a tendency to strengthening of platelets' aggregation, comparison of these results with received ones should be done with great caution. It is connected with the fact that experimental calves in previous investigations were kept in Central Russia in calf-sheds with special heating, and they received substitutes of whole milk and fodder concentrated products.

It is known that activity of neutrophils' aggregation in mammals is provided by locuses' quantity in their glycoprotein receptors' composition. These receptors can connect lectins [30]. It is firmly established that phytogemagglutinin can mostly interact with parts of bD-galactose of glycoproteins, lectin of wheat foetus - with N-acetyl-D-glycosamin и N-acetyl-neuraminic (sialic) acid, and concanavalin A – with N-glycans containing mannose [11]. That is why, the state of lectin stimulated neutrophils’ aggregation of calves is defined by the expression level of receptors’ adhesion. These receptors have such parts in their composition. Taking it into consideration, we can consider that found tendency to growth of neutrophils' aggregation at calves' age of 11-30 days was, evidently, connected with the rise of sensitivity and density of leucocytes' glycoprotein receptors. It happens simultaneously with changing of their composition. Gradual strengthening of lectin - and concanavalin A - induced neutrophils' aggregation in experimental calves was provided by expression increase of adhesion receptors on their surface and by some growth of areas containing N-acetyl-D-glucosamine, N-acetyl-neuraminic acid and mannose. Strengthening increase of aggregation induced by phytogemagglutinin in calves between the 11th and the 30th days of life was provided by a tendency to the rise of areas of glycoproteins, containing bD-galactose [11], in their neutrophils' receptors. Neutrophils’ aggregation was not studied earlier on productive animals and, moreover, on calves. With the help of available literature sources containing information about investigations of a human being, it becomes clear that the role of receptor mechanisms in its realization is great, and that it can be quickly damaged in case of unfavorable environmental and metabolic conditions [11,27].

Noted strengthening of aggregative activity of erythrocytes, platelets and neutrophils in calves during the phase of milk feeding was mostly caused by processes of growth and strengthening of environmental impacts on their background [21]. Sufficient activity of adaptive mechanisms in these conditions keeps the balance of aggregation and disaggregation in calves' blood on the level necessary for optimum of blood supply of internal organs [25].

4. CONCLUSION

During the phase of milk feeding the calves were noted to have a slight tendency to strengthening of lipids' peroxidation in plasma. The calves at the age of 11-30 days were found to have little strengthening of regular blood elements' aggregation.

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Table. The activity of the processes of lipids' peroxidation in plasma and aggregation of blood elements in calves of dairy nutrition

Registrated parameters	Age of calves (n=39, M±m)				
	11 days	**15days**	**20 days**	**25 days**	**30 days**
acyl hydroperoxides, D_{233}/1ml	1.44±0.17	1.46±0.12 P<95%	1.47±0.20 P<95%	1.47±0.15 P<95%	1.49±0.25 P<95%
TBA-active products, umol/l	3.59±0.15	3.63±0.22 P<95%	3.60±0.26 P<95%	3.62±0.19 P<95%	3.64±0.28 P<95%
AOA, %	33.5±0.38	33.3±0.36 P<95%	33.1±0.34 P<95%	32.9±0.29 P<95%	32.4±0.32 P<95%
sum of all the erythrocytes in an aggregate	40.1±0.19	40.2±0.24 P<95%	40.4±0.29 P<95%	40.6±0.25 P<95%	40.9±0.32 P<95%
quantity of aggregates	8.2±0.12	8.2±0.10 P<95%	8.3±0.16 P<95%	8.4±0.19 P<95%	8.4±0.11 P<95%
quantity of free erythrocytes	245.7±2.19	244.2±2.25 P<95%	241.8±2.01 P<95%	242.0±1.90 P<95%	240.4±2.46 P<95%
AP with ADP, s	39.2±0.16	39.0±0.12 P<95%	38.7±0.13 P<95%	38.4±0.10 P<95%	38.1±0.15 P<95%
AP with collagen, s	30.7±0.12	30.5±0.10 P<95%	30.3±0.09 P<95%	30.1±0.11 P<95%	29.7±0.14 P<95%
APwith thrombin, s	52.7±0.15	52.6±0.10 P<95%	52.2±0.16 P<95%	51.7±0.10 P<95%	51.3±0.18 P<95%
APwith ristomicin, s	47.5±0.12	47.2±0.16 P<95%	46.9±0.22 P<95%	46.6±0.26 P<95%	46.2±0.17 P<95%
APwith epinephrine, s	97.8±0.42	97.4±0.36 P<95%	97.1±0.32 P<95%	98.5±0.45 P<95%	98.0±0.34 P<95%
Aggregation neutrophils with lectin, %	14.5±0.16	14.5±0.17 P<95%	14.7±0.15 P<95%	14.9±0.26 P<95%	15.2±0.22 P<95%
Aggregation neutrophils with concanavalin A, %	14.5±0.10	14.6±0.12 P<95%	14.9±0.16 P<95%	15.1±0.11 P<95%	15.5±0.13 P<95%
Aggregation neutrophils with phytogemagglutinin, %	27.1±0.19	27.2±0.23 P<95%	27.4±0.14 P<95%	27.8±0.26 P<95%	28.0±0.21 P<95%

Note: there was found no reliability of indices' changes in comparison with their level at the age of 11 days. We list the values of P – possibility of unmistakable prognosis.

Comment [AL3]: The SEM and exact P values should be added at he and of each row.